Association Between Telomere Length, Specific Causes of Death, and Years of Healthy Life in Health, Aging, and Body Composition, a Population-Based Cohort Study

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Although telomere length (TL) is known to play a critical role in cellular senescence, the relationship of TL to aging and longevity in humans is not well understood. In a large biracial population-based cohort, we tested the hypotheses that elderly persons with shorter TL in peripheral white blood cells have poorer survival, shorter life span, and fewer years of healthy life (YHL). Associations were evaluated using Cox proportional hazard models and linear regression analyses where appropriate. TL (in kilo base pairs) was not associated with overall survival (hazard ratio 1.0; 95% confidence interval 0.9–1.1) or death from any specific underlying cause including infectious diseases, cancer, or cardiac and cerebrovascular diseases. TL, however, was positively associated with more YHL ($\beta = 0.08 \pm 0.04$, $p = .03$). Findings suggest that TL may not be a strong biomarker of survival in older individuals, but it may be an informative biomarker of healthy aging.

Key Words: Telomere—Survival—Life span—Health status—Years of healthy life.

TELOMERES are DNA capping structures protecting the ends of eukaryotic chromosomes whose length often diminishes with aging (1,2). Shortening of telomeres is accelerated in human diseases associated with mutations in telomerase, such as dyskeratosis congenita, idiopathic pulmonary fibrosis, and aplastic anemia (3). Individuals suffering from these conditions show decreased life span coincident with a premature loss of tissue renewal, suggesting that telomerase is rate limiting for tissue homeostasis and organism survival (3). Similar observations have been reported in mice with mutations in genes associated with telomerase (4,5). These observations imply that telomerase activity and telomere length (TL) can directly affect the ability of stem cells to regenerate tissues. Recent data suggest that we age, in part, because our self-renewing stem cells grow old as a result of extrinsic as well as intrinsic factors, such as DNA damage (6). The main implication of this is that stem cell dysfunction as a result of telomere shortening may be one of the mechanisms responsible for organism aging in both humans and mice.

Accumulating evidence implicates DNA damage as a common mediator for both replicative senescence, which is triggered by telomere shortening, and premature cellular senescence induced by various stressors such as oncogenic stress and oxidative stress (7). It has been shown that DNA damage accumulates with age, potentially because of increased production of reactive oxygen species and/or a decline in DNA repair capacity with age (7). On the one hand, mutations or disrupted expression of genes that increases DNA damage often result in premature aging. On the other hand, interventions that enhance resistance to oxidative stress and attenuate DNA damage contribute to longevity.

Previous reports have shown associations of shorter TL with lower survival. Bakaysa and colleagues (8) reported that twins with shorter TL have three times the risk of death compared with their co-twins with longer TL. It has also been reported that in apparently normal elderly people, those with shorter telomeres in blood have an increased risk of overall mortality and death from coronary heart disease and infectious causes (9). In general, older adults have shorter leukocyte TL than younger adults, and women, who on average live longer than men, are reported to have longer telomeres than men (10). We have previously reported the absence of sex differences in leukocyte TL in the Amish, which is consistent with the similar life span observed for Amish men and women who lived until at least age 35 (11). In contrast to the earlier studies, Bischoff and colleagues (12) found no association between leukocyte TL and survival in older individuals. Another study also

found no evidence for monocyte TL predicting age-related morbidity and mortality at ages more than 85 years (13).

There is considerable inconsistency in the current literature on the association between TL and survival in humans. Also, no study on TL and survival has been carried out in the black population. In the present study, we tested the hypothesis that elders with shorter TL have poorer survival and higher mortality rates from specific causes of death using a biracial (blacks and whites) population-based cohort of older individuals from the Health, Aging, and Body Composition (Health ABC) study.

MATERIAL AND METHODS

Study Population

Study participants for this investigation included participants in the Health ABC study, a community-based cohort of 3,075 healthy, well-functioning, men and women aged 70–79 years. To be eligible for participation in Health ABC study, participants had to report no difficulty in walking one-quarter mile (0.5 km) or climbing 10 stairs without resting. Participants were identified from a random sample of white Medicare beneficiaries and all age-eligible black community residents in designated ZIP code areas surrounding Pittsburgh and Memphis. Exclusion criteria included reported difficulty performing basic activities of daily living, obvious cognitive impairment, inability to communicate with the interviewer, intention of moving within 3 years, or participation in a trial involving a lifestyle intervention. All participants gave written informed consent. The institutional review boards at both study sites approved the protocol. Baseline data were collected from 1997 to 1998.

TL Measurement

Average TL in peripheral white blood cells was measured using a validated quantitative polymerase chain reaction (Q-PCR) method (14) which measures the relative average TL in genomic DNA by determining the ratio of telomere repeat copy number to single-copy gene copy number (T/S ratio) in experimental samples relative to a reference sample. The T signal for an experimental DNA sample is the number of nanograms of the reference DNA that matches the experimental sample for copy number of the telomere repeats. The S signal is the number of nanograms of the reference DNA that matches the experimental sample for copy number of the single-copy gene. Experimental samples with T/S >1.0 have longer average TLs than the reference DNA. Experimental samples with T/S <1.0 have shorter average TLs than the reference DNA. The reference DNA is a pooled sample of DNAs from several normal Utah Caucasians, aged 65 years or older. More details on the methods can be found in the supplemental data published online (S1).

All samples were measured in triplicate, and the mean value was used. Results obtained using this method correlate very well with those obtained with the traditional terminal restriction fragment (TRF) length by Southern blot technique (14). In comparison with the TRF method, the Q-PCR method is simple, fast, and less expensive and requires significantly lower amounts of DNA. For this study, each T/S value has been converted to a TL in base pairs by multiplying the T/S value by the known TL of the reference DNA, 4,270 bp. To obtain the TL for the reference DNA, we used the T/S ratios of 64 DNA samples with known mean TRF lengths. The slope of the linear regression line through a plot of T/S ratio (the x axis) versus mean TRF length (the y axis) is the number of base pairs of telomeric DNA corresponding to a single T/S unit. Because the reference DNA has a T/S of 1.0, by definition, this slope is also the average TL of the reference DNA sample, 4,270 bp in our case. Among the 3,075 participants at baseline, 2,880 participants had DNA available and TL was successfully measured in 2,721 individuals.

Study Outcomes

Information used to identify death events included report of death during 6-month contacts with participants; notification of death to Health ABC field centers by proxy, spouse, relative, or friend; hospital record review, review of local newspaper obituaries, death certificates, and if available autopsies. All death events were subsequently confirmed with death certificates. Hospital records, death certificates, informant interviews, and autopsy data were reviewed by the Health ABC Disease Adjudication Committee to adjudicate underlying causes of death. Of the 3,075 participants in this study, 975 (31%) died during the 10-year follow-up period and another 102 (3%) were lost to follow-up. The mean follow-up time was 8.2 ± 2.3 years.

Years of healthy life (YHL) is a secondary outcome measure of successful aging that integrates self-perceived functional status and duration of survival. Standard information on self-rated health status (excellent/very good/good/fair/poor) was used to estimate YHL as described previously (15–17). Briefly, excellent/very good/good were coded as 1 for "healthy" and fair/poor/dead were coded as 0 for "not healthy." To address the problem of missing data due to death in follow-up studies, we transformed self-rated health or physical performance to new variables that incorporate a value for death. The length of healthy life for n years of follow-up was estimated as $H0/2 + H1 + H2 + H3 + ... + Hn/2$. $H0$ stands for health status at Year 0, that is, baseline, $H1$ stands for health status during Year 1, and Hn stands for health status during Year n. Health status can be assigned the value of 0 for not healthy or 1 for healthy. The values for the first and the last years were weighed down (50%) to account for the fact that health status information did not represent the entire year.

Statistical Analyses

We constructed a proportional hazard model (Cox regression) to compute the hazard ratio (HR) for each specific cause

Table 1. Baseline Characteristics of the Study Population by Race and Sex

Traits, $M \pm SD$ or Number (%)	Whites		Blacks	
	Men (939)	Women (855)	Men (552)	Women (729)
Recruitment site				
Pittsburgh	476 (51)	459 (54)	276 (50)	337 (46)
Memphis	463 (49)	396 (46)	276 (50)	392 (54)
Age (y)	$73.9 \pm 2.9^*$	73.6 ± 2.8	73.5 ± 2.8	73.4 ± 2.9
TL (kbp)	$4.6 \pm 1.2^*$	5.0 ± 1.3	$4.6 \pm 1.1^*$	5.0 ± 1.2
Number of death	$312 (34)^*$	186 (22)	$254 (48)^*$	223 (32)
Life span (y)[†]	80.4 ± 3.7	80.2 ± 3.7	79.3 ± 4.0	79.7 ± 4.0
YHL (y)	5.5 ± 2.2	5.7 ± 2.0	$4.1 \pm 2.6^*$	4.5 ± 2.5

Notes: TL = telomere length; YHL = years of healthy life.
  *Men vs women, $p < .05$.
  [†]In 975 individuals who died during 10 years of the follow-up period.

of death. Participants alive after 10 years of assessment were censored. We tested the proportional hazard assumption using the log–log survival curve and the goodness-of-fit test. Potential confounders included in the regression equation were age, race, sex, and the study site. Additionally, we adjusted for assay plate by introducing plate number as a random factor in our models. Plates were run on different days, and batch variation is a source of random error. We also evaluated interactions between TL, race, and sex. HR and 95% confidence interval (CI) were estimated. In order to investigate the association of TL with survival on a linear scale, we used general linear models to compare mean TL adjusted for age, sex, race, and site, for each specific cause of death. All analyses were done using SPSS 14 for Windows, Chicago, IL.

RESULTS

Table 1 summarizes the baseline characteristics of the study population stratified by race and sex. In both black and whites, women had significantly longer TL, on average 400 bp longer, compared with men ($p < .001$). We observed a marginal difference in mean TL by race (TL was 4.77 ± 1.3 kbp in blacks vs 4.87 ± 1.1 kbp in whites, $p = .05$). There were significantly more deaths in men compared with women for both races ($p < .001$) but more deaths in blacks compared with whites ($p < .05$). There was no difference in life span between men and women ($p = .08$). Participants in this study who had died at the end of our follow-up period ($n = 975$) lived for 80 years on average. There was no significant difference in the mean YHL between white men (5.5 ± 2.2) and women (5.7 ± 2.0, $p = .1$); however, black men had significantly fewer YHL (4.1 ± 2.6) compared with black women (4.5 ± 2.5, $p < .01$). Also blacks had significantly ($p < .01$) fewer YHL compared with whites (4.3 ± 2.5 vs 5.6 ± 2.1, $p < .01$).

There was no significant association between TL and overall mortality (HR 1.0; 95% CI 0.9–1.1). The relationship between TL and specific cause of death is presented in Table 2. In this analysis, TL was modeled as a continuous trait (in kilo base pairs). There was no significant effect modification by race or sex. Therefore, results are shown for the entire cohort. We found that for each decrease in TL of 1 kbp, risk of dying from infections or pneumonia as an underlying cause increased by 20% and 30%, respectively, although the p values were not statistically significant ($p = .3–.4$). Mortality due to cancer and cardio- and cerebrovascular diseases was not observed to be higher with shorter TL. Likewise, shorter TL was not associated with increased risk of dying from renal or respiratory diseases.

To investigate if our observations on a logistic scale differed on a linear scale, we computed mean TL (adjusted for age and sex) by specific cause of death (see Table 3). There was no significant difference in the mean TL between individuals who died of renal disease (4.7 ± 0.3 kbp) compared with those alive (4.9 ± 0.3 kbp). Individuals who died of infections (sepsis, pneumonia) had in general shorter TL compared with individuals alive. Except for cancer as cause of death, the means of TL were generally higher in individuals alive compared with those who died. The results presented here in which TL was treated as a continuous trait did not differ much from the results when TL was treated as a categorical trait, comparing, for example, tertiles or quartiles.

Interestingly, we observed a stronger association between TL and self-reported health status. Figure 1 shows the mean TL (adjusted for age, sex, race, and recruitment site) by self-reported health status at baseline. We observed that TL was significantly higher in individuals who reported "excellent" or "very good" health status compared with individuals who reported "fair" or "poor" health status ($p < .02$). There was a significant trend between the TL and self-reported health status (p for trend = .01). Moreover, TL was also significantly associated with YHL based on 8 years of cumulative information on self-reported health status. We observed that the mean YHL was increased with longer TL ($\beta = 0.08 \pm 0.04$, $p < .03$), in both men and women.

DISCUSSION AND CONCLUSION

In a large population-based cohort study of older individuals, we investigated the association between TL and

Table 2. Association Between Telomere Length (in kilo base pairs) and Survival by Specific Underlying Causes of Death

Cause of Death	Event/Censored	HR (95% CI)	p
Cancer	239/1,783	1.0 (0.9–1.2)	.5
CHD	189/1,783	1.0 (0.9–1.1)	.8
CVD	69/1,783	1.0 (0.8–1.2)	.9
Pneumonia	9/1,783	0.7 (0.4–1.4)	.3
Infection	23/1,783	0.8 (0.5–1.1)	.4
Renal disease	22/1,782	0.9 (0.6–1.3)	.5
Dementia	43/1,782	1.0 (0.7–1.3)	.8
Respiratory disease	39/1,783	1.0 (0.7–1.3)	.8

Notes: CHD = coronary heart disease; CI = confidence interval; CVD = cerebrovascular disease; HR = hazard ratio.

Figure 1. Adjusted mean telomere length (kilo base pairs) by self-reported health status at baseline. Bars represent 1 *SE*.

survival, life span, YHL, and mortality due to specific underlying causes of death. We found that longer TL measured in peripheral white blood cells at baseline was associated with longer YHL across the entire sample of black and white men and women. Longer TL also was associated with reduced odds of death from infectious diseases at follow-up. We did not observe an association between TL and overall survival or life span.

Telomere shortening has been associated with many age-related diseases (18–21). In our study, there was a nonsignificantly increased risk of dying from infectious diseases in individuals with shorter TL. Cawthon and colleagues (9) reported a higher mortality rate from infectious diseases in individuals with shorter telomeres. Also a very recent experimental study (22) has demonstrated that repeated *Salmonella* infections cause telomere attrition in white blood cells.

There is conflicting evidence in the literature on the association between TL, survival, and life span. Some studies have reported a positive association between TL and survival in elderly people (23), demented individuals (24), and twins (8). On the other hand, some studies reported no apparent relationship between TL and survival in elderly people (12) or centenarians (13). Our study in elderly people concurs with reports of no association between TL and survival. A number of reasons may account for these seem-

ingly inconsistent reports in the literature. One possible explanation is the difference in methods of TL measurement across these various studies. We measured TL by Q-PCR method. This method does not measure the subtelomeric segment (estimated to range from 2,500 to 6,000 bp) contrary to the Southern blot method used by earlier studies. Both methods correlate well with each other ($r = 0.7$) (14), but the Q-PCR method appears to have a larger coefficient of variation (5.8%) (14) than the Southern blot method (0.9%–12%) (23,25,26), which may reduce power to detect small effects of TL. Also, this study and several other studies showing no association between TL and survival have been carried out in older populations. It is possible that the effect of TL on survival varies by age. There are other possible explanations for the lack of association between TL and survival. First, despite a relatively large sample size, we still did not have sufficient power to detect a small HR (e.g., 1.1) in our analyses of survival. Second, the relative old age range in our study population may be more likely to be affected by survival bias. Individuals with aging-related disorders or those who would have died prior to reaching age 70 would be selected out in an elderly population such as ours, leaving the "survivors" with relatively longer telomeres and a narrower range in TL. The third possible explanation is biologic. Cellular senescence may contribute to organismal aging by altering patterns of gene expression in aged cells (27,28). Therefore, the informativeness of TL measured at an older age may be more limited compared with TL measured at a younger age.

To the best of our knowledge, our study is the first to examine and report an association between TL and YHL. We found that longer TL was significantly associated with an increase in the number of YHL, after adjusting the effects of sex and race. Such an observation may suggest that TL measured in white blood cells is indicative of the degree of overall well-being in older individuals. Indeed, a recent study comparing 19 healthy centenarians versus 19 unhealthy centenarians has shown that longer TL was associated with better health (29). This study although

Table 3. Adjusted $M \pm SD$ of TL by Specific Underlying Cause of Death

Cause of Death	N	TL (kbp), $M \pm SD$
Cancer	239	4.9 ± 1.2
CHD	189	4.8 ± 1.3
CVD	69	4.8 ± 1.1
Renal disease	22	4.7 ± 1.1
Infection*	23	4.5 ± 1.0
Pneumonia	9	4.4 ± 1.1
Respiratory disease	39	4.8 ± 1.2
Dementia	43	4.8 ± 1.4

Notes: Mean TL in participants alive = 4.9 ± 1.2 kbp. Adjusted for age, sex, race and recruitment site. CHD = coronary heart disease; CVD = cerebrovascular disease; TL = telomere length.
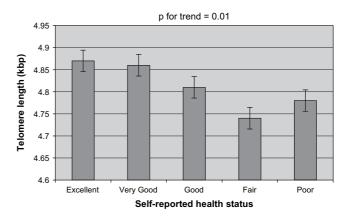
*Infection includes sepsis and pneumonia.

limited in sample size also used the Q-PCR method, and their findings are similar to ours. In fact, even with a less sensitive method, our study in a very large sample is sufficiently powered. Our results confirm this previous report and further show a trend in mean TL by self-reported health status.

Because this study was carried out in a biracial population of blacks and whites, the findings are potentially applicable to multiple other populations. Another strength is the population-based design, with complete follow-up on most individuals and information on specific causes of death. There are a few limitations to the current study. For many causes of death, the sample size was low and the power to detect an association was very limited.

In conclusion, we did not find any evidence of association between TL and overall survival or between TL and specific causes of death. We also report for the first time that longer TL is associated with self-reported health status and greater YHL. Findings suggest that TL, although not a strong biomarker of survival in older individuals, may be an informative biomarker of healthy aging.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at: http://biomed.gerontologyjournals.org/

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